MUC1-C ACTIVATES EZH2 EXPRESSION AND FUNCTION IN HUMAN CANCER CELLS

Hasan Rajabi, Masayuki Hiraki, Ashujit Tagde, Maroof Alam, Audrey Bouillez, Camilla L. Christensen, Mehmet Samur, Kwok-Kin Wong and Donald Kufe*

Dana-Farber Cancer Institute
Harvard Medical School
Boston, MA 02215

***Corresponding Authors:** Donald Kufe, 450 Brookline Avenue, DA830, Boston, Massachusetts, 02215, 617-632-3141 Tel., 617-632-2934 Fax, donald_kufe@dfci.harvard.edu

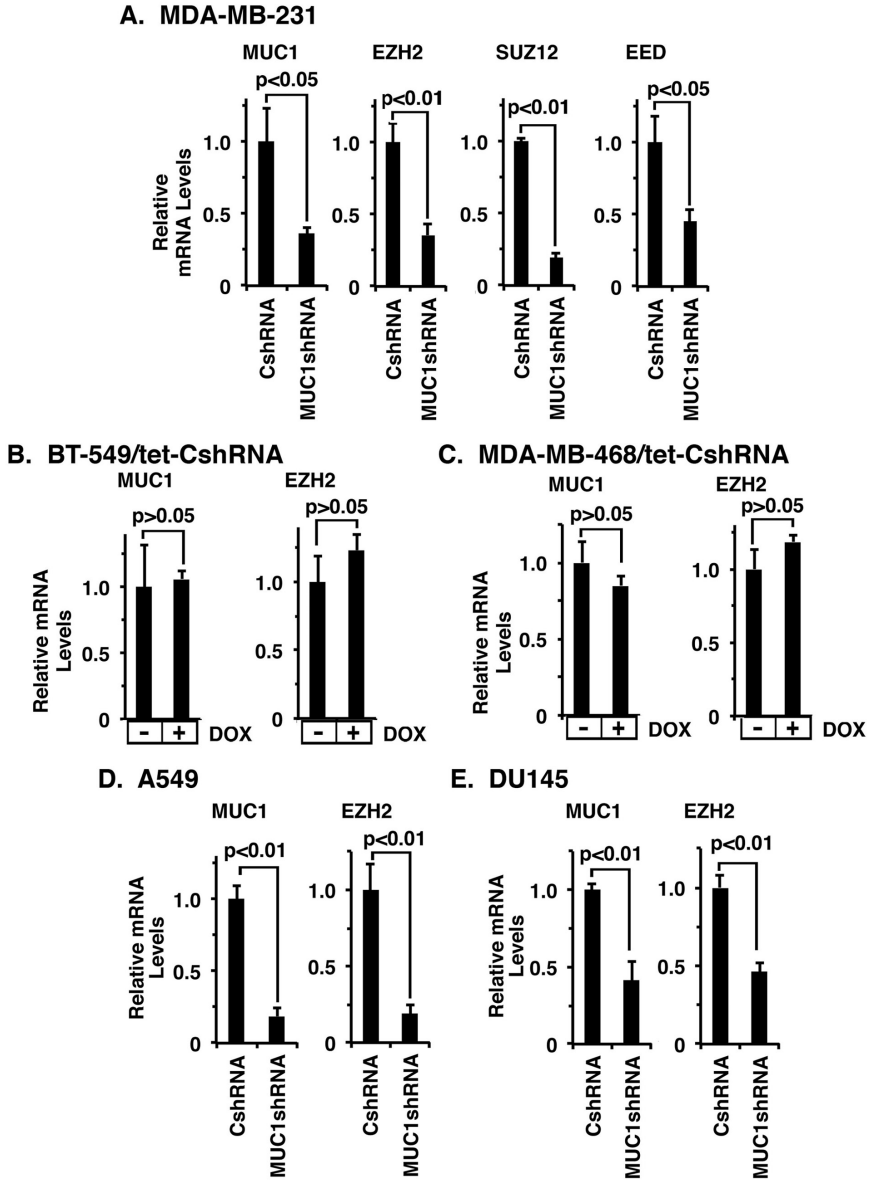


Figure S1. MUC1-C regulates EZH2 expression in diverse types of carcinoma cells. Relates to Figure 1. A. MDA-MB-231 cells stably expressing a control scrambled shRNA (CshRNA) or a MUC1shRNA were analyzed for MUC1, EZH2, SUZ12 and EED mRNA levels by qRT-PCR. The results (mean±SD) are expressed as relative mRNA levels compared to that obtained for the CshRNA cells (assigned a value of 1). B and C. BT-549 (B) and MDA-MB-468 (C) cells were stably transduced to express a tetracycline-inducible control scrambled shRNA (tet-CshRNA). Cells treated with 200 ng/ml DOX for 4 d were analyzed for MUC1 and EZH2 mRNA levels by qRT-PCR. The results (mean±SD) are expressed as relative mRNA levels compared to that obtained for control DOX-untreated cells (assigned a value of 1). D and E. A549 (D) and DU145 (E) cells stably expressing a control scrambled shRNA (CshRNA) or a MUC1shRNA were analyzed for MUC1 and EZH2 mRNA levels by qRT-PCR. The results (mean±SD) are expressed as relative mRNA levels compared to that obtained for the CshRNA cells (assigned a value of 1).

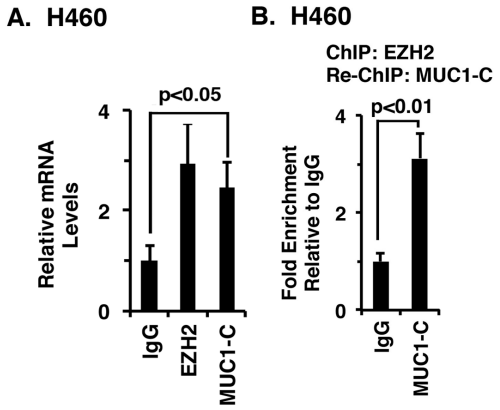


Figure S2. MUC1-C occupies the *CDH1* promoter in a complex with EZH2. Relates to Figure 6. A. Soluble chromatin from H460 cells was precipitated with anti-EZH2, anti-MUC1-C or a control IgG. The final DNA samples were amplified by qPCR with primers for the *CDH1* promoter (Table S2). The results (mean±SD of three determinations) are expressed as the relative fold enrichment compared with that obtained with the IgG control (assigned a value of 1). B. In the re-ChIP analysis, anti-EZH2 precipitates were released and re-immunoprecipitated with anti-MUC1-C or a control IgG. The final DNA samples were amplified by qPCR with primers for the *CDH1* promoter. The results (mean±SD of three determinations) are expressed as the relative fold enrichment compared with that obtained with the IgG control (assigned a value of 1).

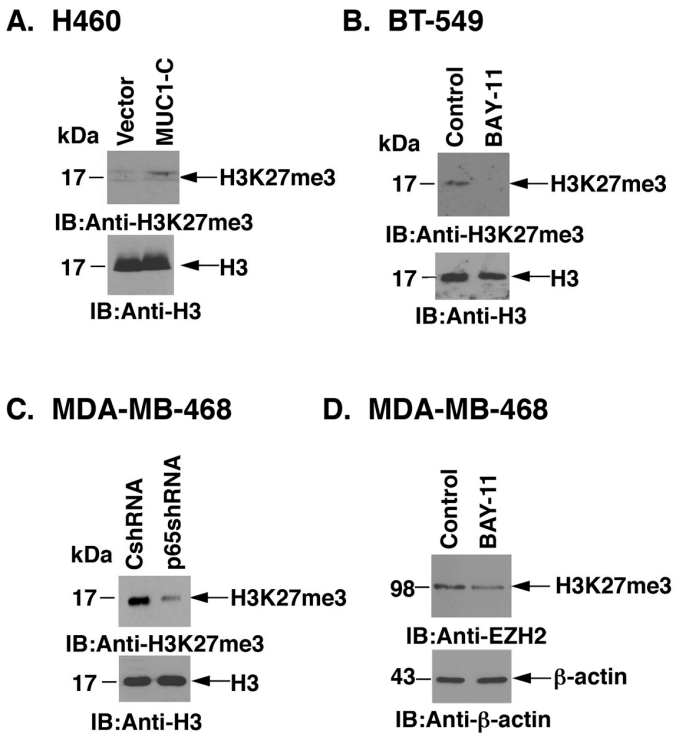


Figure S3. MUC1-C and NF-κB p65 drive H3K27 trimethylation. Relates to Figure 6. A. Lysates from H460/vector and H460/MUC1-C cells were immunoblotted with the indicated antibodies. B. Lysates from BT-549 cells treated with 5 μM BAY-11-7085 or vehicle control for 48 h were immunoblotted with the indicated antibodies. C. Lysates from the MDA-MB-468/vector and MDA-MB-468/p65shRNA cells were immunoblotted with the indicated antibodies. D. Lysates from MDA-MB-468 cells treated with 5 μM BAY-11-7085 or vehicle control for 48 h were immunoblotted with the indicated antibodies.

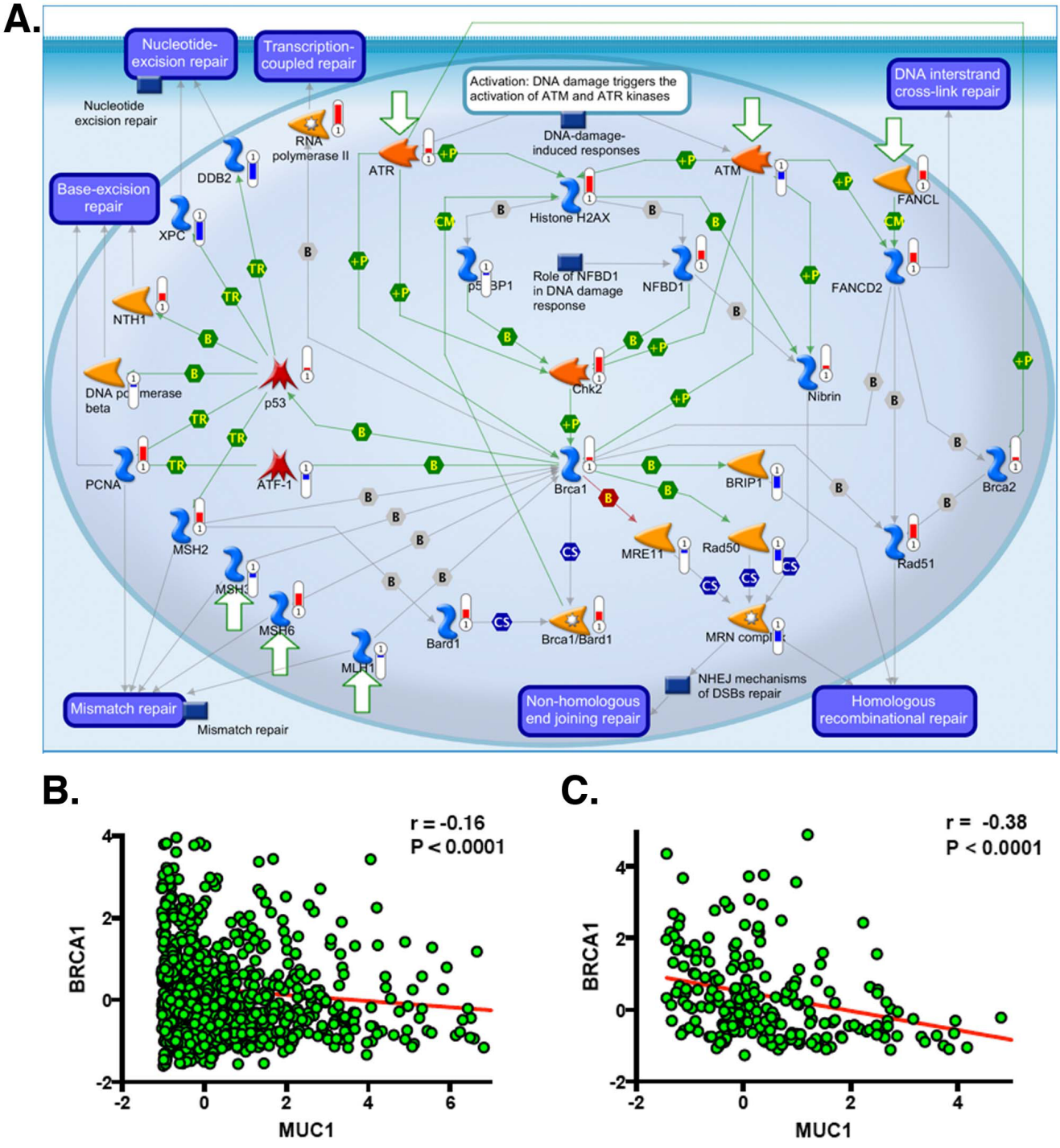


Figure S4. MUC1 expression correlates negatively with that of BRCA1. A. RNA-seq data from H460/CshRNA and H460/MUC1shRNA cells was analyzed using MetaCore for enrichment. B and C. *MUC1* and *BRCA1* gene expression data from TCGA datasets (cBioPortal) of breast (B; n=817) and lung (C; n=230) was assessed using the Spearman's correlation coefficient, where $p<0.05$ was considered as statistically significant.

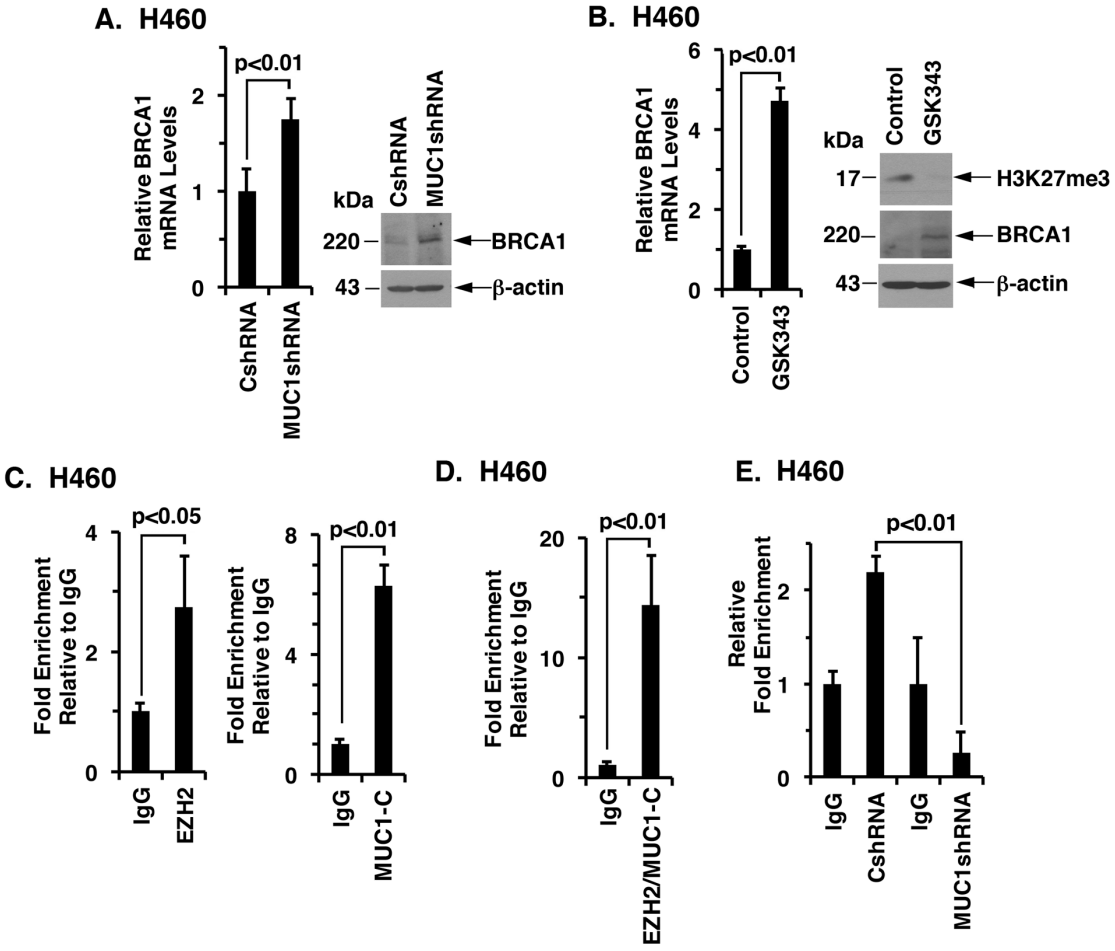


Figure S5. Targeting MUC1-C and EZH2 induces BRCA1 expression in H460 cells. A. H460/CshRNA and H460/MUC1shRNA cells were analyzed for BRCA1 mRNA levels by qRT-PCR. The results (mean±SD) are expressed as relative mRNA levels compared to that obtained for the CshRNA cells (assigned a value of 1)(left). Lysates were immunoblotted with the indicated antibodies (right). B. H460 cells treated with vehicle control or 10 μM GSK343 for 72 h were analyzed for BRCA1 mRNA levels by qRT-PCR. The results (mean±SD) are expressed as relative mRNA levels compared to that obtained for the Control cells (assigned a value of 1)(left). Lysates were immunoblotted with the indicated antibodies (right). C. Soluble chromatin from H460 cells was precipitated with anti-EZH2 (left), anti-MUC1-C (right) or a control IgG. D. In the re-ChIP analysis, EZH2 precipitates were released and re-immunoprecipitated with anti-MUC1-C and a control IgG. E. Soluble chromatin from H460/CshRNA and H460/MUC1shRNA cells was precipitated with anti-H3K27me3 or a control IgG. The final DNA samples were amplified by qPCR with primers for the *BRCA1* promoter. The results (mean±SD of three determinations) are expressed as the relative fold enrichment compared with that obtained with the IgG control (assigned a value of 1).

Table S1. Primers used for qRT—PCR.

MUC1 qRT-F	AAAGCGATGGCGATTGGG
MUC1 qRT-R	CTCACCAGCCCAAACAGG
EZH2 qRT-F	ATTTCGTAGGAGGGAGCAAAG
EZH2 qRT-R	TGGGCCTGCTACTGTTATTG
SUZ12 qRT-F	GCAGCTTACGTTTACTGGTTTC
SUZ12 qRT-R	TGAGTTTGGTGATGGCTTATCT
EED qRT-F	TAAGGGCACGTAGAGCATTTAG
EED qRT-R	TGAGCAGGAAGACAGTACAAAG
NF-κB-p65 qRT-F	TGAGCCCACAAAGCCTTATC
NF-κB-p65 qRT-R	ACAATGCCAGTGCCATACA
E-cadherin qRT-F	GAACAGCACGTACACAGCCCT
E-cadherin qRT-R	GCAGAACTGTCCCTGTCCCAG
BRCA1 qRT-F	CCTTCTACTGTCCTGGCTACTA
BRCA1 qRT-R	CAGATTTCCAAGGGAGACTTCA
GAPDH qRT-F	CCATGGAGAAGGCTGGGG
GAPDH qRT-R	CAAAGTTGTCATGGATGACC

Table S2. Primers used for ChIP qPCR.

ChIP-qPCR primers *EZH2* promoter	
qF	AAAGCGATGGCGATTGGG
qR	TCCACTGCCTTCTGAGTCC
ChIP-qPCR primers *EZH2* intron-1	
qF	GCCTCAAGTCTCCTTTGTGT
qR	CCCACCAACTTGTGTCTGT
ChIP-qPCR primers for *CDH1* promoter	
qF	CTTTCTGATCCCAGGTCTTAGTG
qR	TAGGGTCTAGGTGGGTTATGG
ChIP-qPCR primers for *BRCA1* promoter	
qF	AAAGCGATGGCGATTGGG
qR	TCCACTGCCTTCTGAGTCC
ChIP qPCR primer *GAPDH* promoter	
qF	TACTAGCGGTTTTACGGGCG
qR	TCGAACAGGAGGAGCAGAGAGCGA

Figure 1C L

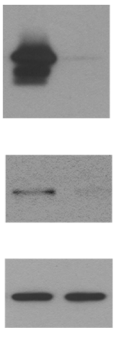


Figure 1C R

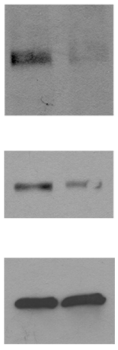


Figure 1D

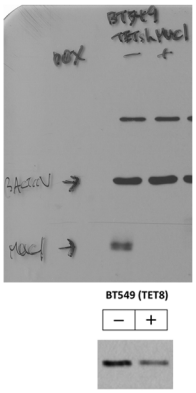


Figure 1E

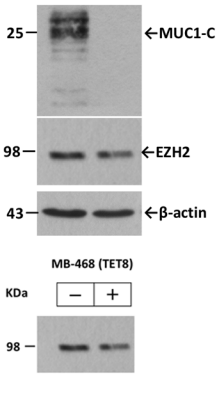


Figure 2B left

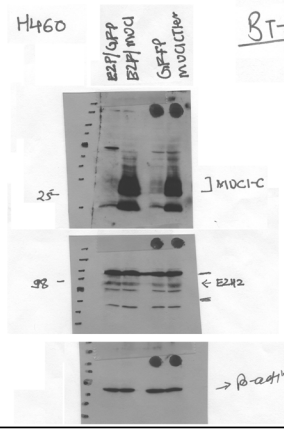


Figure 2B right

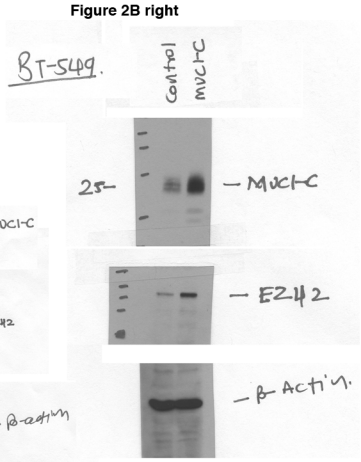


Figure 2D

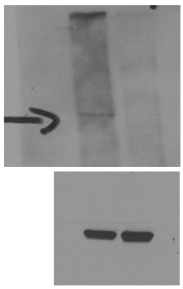


Figure 2E

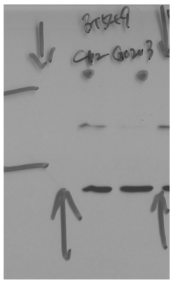


Figure 2F

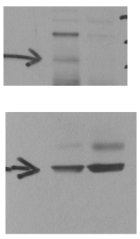


Figure 3D left p-pRB

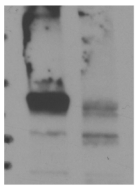


Figure 3D right p-pRB

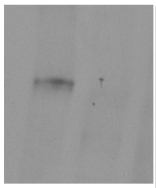


Figure 4C

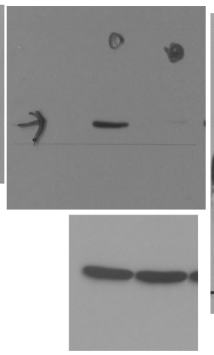


Figure 5D

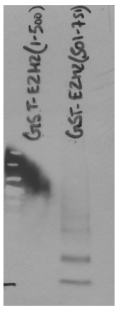
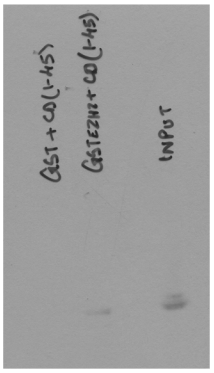


Figure 5E left



5E right

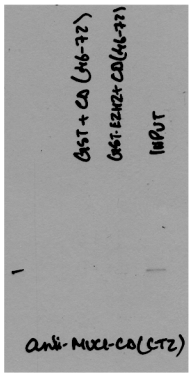


Figure 5F

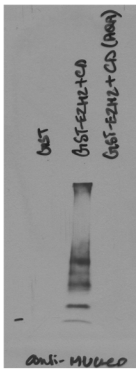


Figure 5G

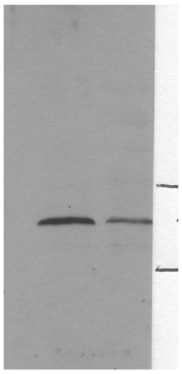
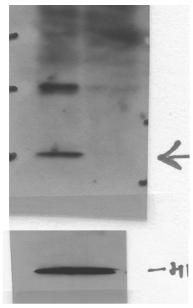
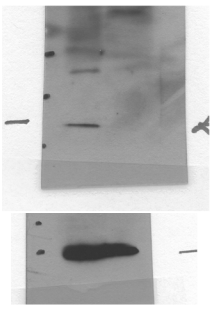


Figure 6A



6B



6C

6D

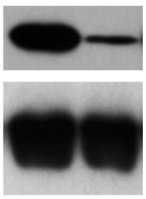


Figure 7A

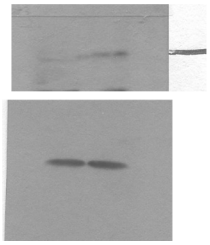


Figure 7 B